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A STUDY ON THE INNERVATION OF THE MACULA CRIBRIFORMIS IN THE DIAPHRAGMATIC PERITONEUM OF THE RABBIT

by

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1. INTRODUCTION

Since von RECKLINGHAUSEN (1863), many authors have observed that foreign particles injected into the peritoneal cavity are mainly absorbed through the lymphatic vessels in the diaphragm, but none of them has definitely demonstrated the course by which these foreign particles reach the lymphatic vessels.

Recently T. KIHARA et al demonstrated that the subdiaphragmatic peritoneum has a sieve-like structure composed of collagen and reticular fibers, i. e. the macula cribriformis, which is the primary course in the absorption of foreign particles in the peritoneal cavity. KIHARA found it to be one of the pre-lymphvascular fluid path, and he demonstrated the same structure in the subendothelial connective tissue of the omentum, the parietal pleura and the mediastinal pleura.

The author, in his neuro-histological study on ileocecal lymph nodes, found nerve fibers in the secondary follicles.

Considering the analogous function and the similarity of the tissue elements composing the macula cribriformis with that of the lymph nodes, the author proceeded to study the nervous structures in the macula and their physiological significance.

2. HISTOLOGICAL OBSERVATIONS ON THE NERVE DISTRIBUTION IN THE MACULA CRIBRIFORMIS OF THE DIAPHRAGMATIC PERITONEUM

Materials and methods

Fresh diaphragms of adult rabbits were used.

After fixation of materials in 10% neutral formol solution for at least 2 months, small pieces of the diaphragm were cut out. From the surface of the thoracic side, the pleura, the muscular and the tendinous fibers were carefully dissected with a small pincette and a thin peritoneal membrane was prepared. The peritoneal membrane was spread over a slide for microscopic observation.

BIELSCHOWSKY's silver impregnation and EHRLICH's acid hematoxyline staining were used on these materials, the former for the axis-cylinders and the letter for the myelin sheaths.

Furthermore, WALLERIAN degeneration was observed in the materials, of which

the vagus or the phrenic nerve had previously been sectioned.

The vagus nerve was cut distal to the nodular ganglion on one side of the neck and the materials were removed 5, 6, 7 or 10 days after the operation.

The phrenic nerve was cut on one or both sides of the neck and the diaphragms were removed 5, 6, 7, 8 or 14 days after the nerve operation.

Microscopic observation

On the materials with well impregnated BIELSCHOWSKY's method modified by SUZUKI, the fine structure of the macula cribriformis was observed together with the nerve fibers distributed in it.

The macula cribriformis in the subendothelial connective tissue of the diaphragmatic peritoneum was most clearly developed at the border between the tendinous portion and the muscular portion of the diaphragm, but even in this area the sieve-like structure could not be found under the large veins. The maculae in the peritoneum covering the tendinous portion of the diaphragm were distributed regularly in parallel with tendon bundles and extended from the border toward the center of the tendinous diaphragm, while those in the muscular portion of the diaphragm were poorly developed and spindle-shaped (Fig. 1-3).

The collagen fibers composing the macula had a greater affinity for silver than did other connective tissue fibers. Ramifying and anastomosing with each other, they formed a large network, and within the interstices fine fibers formed smaller networks (Fig. 4).

Some large blood vessels ran along the border between the tendinous and the muscular portion of the diaphragm, which gave rise to branches toward the tendinous portion and the muscular portion. Many of these large blood vessels coursed independently of the arrangement of the macula cribriformis, while some small blood vessels or capillaries were found along the thick collagen fibers of the macula (Fig. 5-6).

Many of the nerve fibers in the diaphragmatic peritoneum were found along the course of the blood vessels. They were observed as nerve fibers of varying thickness or as nervous syncytium surrounded by a nervous plasmodium. They ramified along with the blood vessels and ran through the subperitoneal layer (Fig. 7-11), except for a few nerve fibers which took their course separately from the blood vessels (Fig. 12-13).

In the macula cribriformis, the nerve fibers usually ran along the collagen fibers composing the macula. Many of them ramified along with the branching of collagen fibers and took a complicated course.

Some thinner nerve fibers ended in the collagen fibers, but no special end-apparatus was observed there. The nerve fibers were not traced to the fine network of the macula cribriformis.

Many of the nerve elements in the macula consisted of fine fibers and nervous syncytium, and SCHWANN's nuclei were found in places along their course. The syncytial structure and SCHWANN's nuclei distinguished them as nerve elements and allowed differentiation from other tissue fibers (Fig. 14-22).

Degeneration experiments of nerve fibers in the macula cribriformis

The author studied the root of the nerve fibers distributed in the macula cribriformis by means of WALLERIAN degeneration.

One specimen of these, on which vagotomy had been performed 10 days before, showed partial severance of a part of a myelinated fiber around the macula cribriformis, but no sign of degeneration was found in any of the other specimens (Fig. 23-24).

The diaphragms which were removed 5, 6, 7, 8 & 14 days after section of the phrenic nerve showed no degenerated myelinated fibers around the macula (Fig. 25).

From these results, the author could not find any continuity of nerve fibers in the macula cribriformis and its surroundings with the vagus and phrenic nerve.

Discussion

DOGIEL (1902) stained the nerve fibers in the parietal peritoneum of human beings and other mammals with methylene blue and came to the conclusion that those nerve fibers formed a nerve plexus with the appearance of a network in the serous and subserous layers of the peritoneum, and the myelinated fibers ended in encapsulated or non-capsulated end apparatuses, while the non-myelinated fibers were distributed along the blood vessels.

There are many reports on the nerve distribution of the lymphatic apparatuses. ITO (1944) observed nerve fibers in the lymph nodes and PEYER's plaques ending in the walls of blood vessels and reticular tissues.

However, no description can be found of the nerve distribution in the macula cribriformis composing the pre-lymphvascular fluid path (KIHARA).

The author successfully demonstrated nerve fibers distributed along the collagen fibers of the macula cribriformis.

Many of them had branches just at the knots of collagen fibers and often had a complicated course, but at least some of them seemed to end in the collagen fibers.

The author considered, therefore, that they were nerve fibers playing a role in controlling the function of the macula cribriformis.

3. EXPERIMENTS ON THE INFLUENCE OF UNILATERAL VAGOTOMY, SYMPATHECTOMY AND SECTION OF THE PHRENIC NERVE ON THE ABSORPTION OF FOREIGN PARTICLES THROUGH THE MACULA CRIBRIFORMIS IN THE DIAPHRAGMATIC PERITONEUM

Having observed the distribution of nerve fibers in the macula cribriformis in the diaphragmatic peritoneum, other experiments were done to determine what influence unilateral vagotomy, sympathectomy or section of the phrenic nerve has on the absorption of foreign particles through the macula.

Materials and methods

Adult rabbits weighing about 2kg were used.

The experimental animals were classified into 4 groups;

1st group·····control.
2nd group·····the vagus nerve was sectioned on one side of the neck.
3rd group·····the phrenic nerve was sectioned on one side of the neck.
4th group·····the sympathetic nerve was blocked with 2% novocain solution from 4T to 8T on one side.

After a certain interval 25cc per kg of India ink was injected into the peritoneal cavity through a thin vinyl tube inserted into the upper abdominal wall in the midline. 30 minutes after injection, when the absorption of India ink through the macula was at its height, the animals were killed and the deposition of the ink in the subpleural diaphragmatic lymphatic vessels was examined macroscopically. Some of them were examined within 10 minutes after injection.

The darkness and the thickness of the subpleural diaphragmatic lymphatics of both sides were compared with each other to examine the extent of absorption through the macula cribriformis of both halves of the diaphragmatic peritoneum.

Table 1

method of operation	animal No.	side of section of nerve	interval from operation to injection	interval from injection to death	extent of India ink filling in the subpleural lymphatics on both halves of the diaphragm
lst group control	33	···	···	30 minutes	left > right
	34	···	···	〃	left < right
	40	···	···	〃	left ≒ right
	41	···	···	〃	left < right
2nd group unilateral vagotomy	22	right	2 days	8 minutes	left ≒ right
	7	〃	5 days	30 minutes	left < right
	2	〃	6 days	〃	left ≒ right
	4	〃	7 days	〃	left < right
	6	left	8 days	〃	left < right
3rd group unilateral phrenicotomy	30	right	2 days	30 minutes	left ≒ right
	31	〃	2 days	〃	left > right
	32	〃	2 days	〃	left < right
	24	〃	3 days	〃	left > right
	17	〃	4 days	10 minutes	left ≒ right
	19	left	5 days	〃	left ≒ right
	5	right	7 days	30 minutes	left < right
	16	〃	7 days	8 minutes	left > right
4th group unilateral sympathetic block	21	right	injected immediately after the sympathetic block	30 minutes	left ≒ right
	26	〃		〃	left < right
	42	〃		〃	left > right
	43	〃		〃	left ≒ right

Experimental results

Results are shown in Table 1 and Figures 26·····31.

In the control animals of the lst group, the India ink was taken into the subpleural diaphragmatic lymphatics of both halves to the same degree only in one case. In 2 other cases the India ink filled the lymphatic vessels somewhat more on the right half than on the left, and in another case more on the left half than on the right. Thus the absorption in the both halves of the diaphragmatic peritoneum seemed usually to be unequal.

In the 2nd, 3rd and 4th groups, about half of the cases in each group took the ink in the subpleural diaphragmatic lymphatics of both halves to the same degree,

while the other half of the cases took somewhat more ink on one side or the other.

These results gave no conclusion that denervation or nerve blockage of the diaphragmatic peritoneum causes any change in the absorption through the macula cribriformis.

Discussion

HIGGINS (1930) injected graphite particles into the peritoneal cavity of dogs and observed the time until they appeared in the lymphatic vessels of the diaphragm as well as the quantity of particles absorbed.

MORRIS (1953) injected erythrocytes labeled with P^{32} into the peritoneal cavity of rats and observed their migration.

These two authors agreed that the absorption of foreign particles from the peritoneal cavity through the diaphragmatic peritoneum was interfered with soon after the section of the phrenic nerve.

In the author's experiments, unilateral section of the phrenic nerve did not result in any definite change in the absorption of the foreign particles through both halves of the diaphragmatic peritoneum, nor did unilateral vagotomy or sympathectomy.

The author's results may be of course, because different degrees of absorption on each half are shown even in normal diaphragmatic peritoneum.

4. SUMMARY AND CONCLUSION

1) With BIELSCHOWSKY-SUZUKI's silver impregnation and EHRLICH's hematoxyline staining, the author demonstrated nerve fibers in the macula cribriformis of the diaphragmatic peritoneum, which is regarded as a pre-lymphvascular fluid path.

2) Section of the vagus nerve or the phrenic nerve did not cause marked degeneration of nerve fibers in the macula cribriformis or its surrounding area in the diaphragmatic peritoneum.

3) Uuilateral section of the vagus nerve or the phrenic nerve did not result in any change in absorption in the denervated half of the diaphragmatic peritoneum. The same was true when the sympathetic block was done on one side.

4) The macula cribriformis in the diaphragmatic peritoneum has nerve fibers, but in regard to its function, the present study cannot demonstrate any relation with the absorption of foreign particles in the peritoneal cavity through the macula.

I am very grateful to Assistant Professor Ch. KIMURA who gave me constant help and good suggestions throughout this work.

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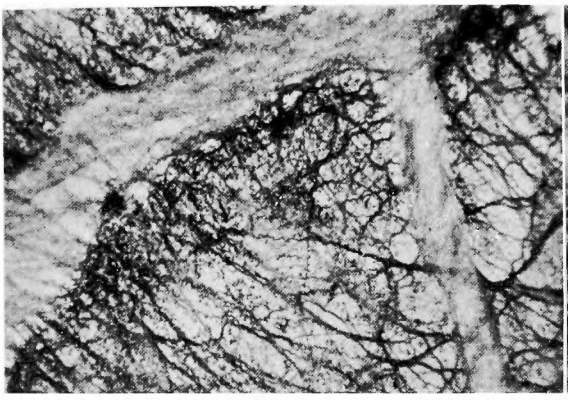

Fig. 1 The macula cribriformis in the border between the tendinous and the muscular portion of the diaphragm. (B-stain) ×40

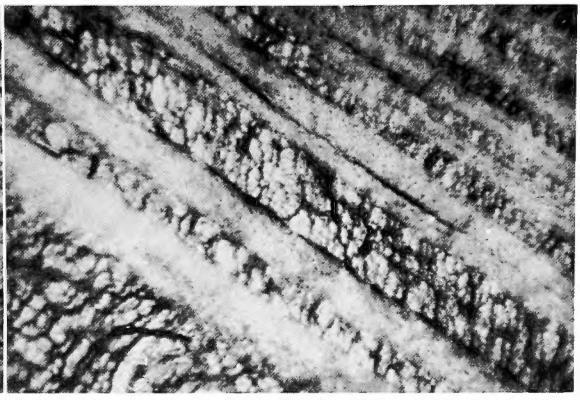

Fig. 2 The macula cribriformis in the tendinous portion of the diaphragm. (B-stain) ×40

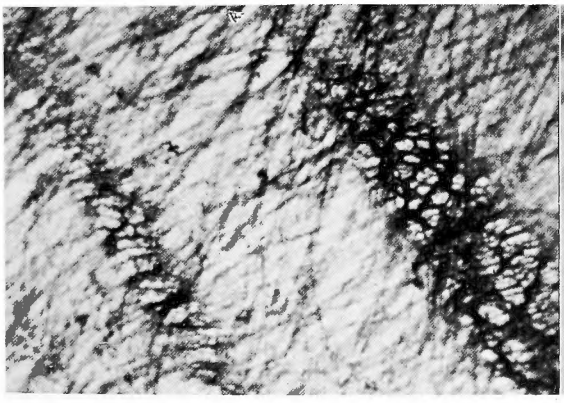

Fig. 3 The macula cribriformis in the muscular portion of the diaphragm. (B-stain) ×40

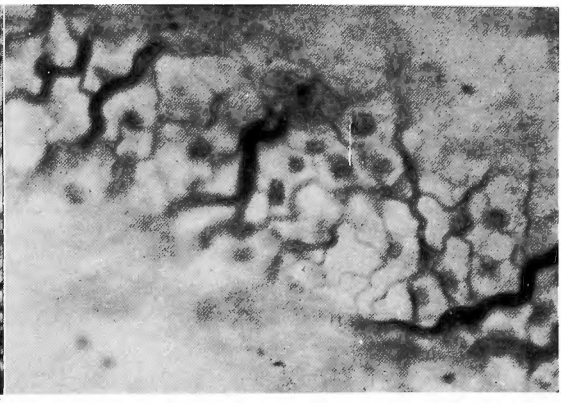

Fig. 4 Fine structure of the macula cribriformis. (tendinous portion) (B-stain) ×400

Fig. 5 Blood vessels distributed in the diaphragmatic peritoneum. (B-stain) ×40

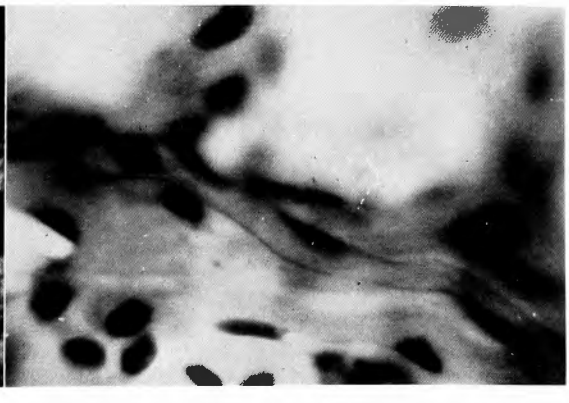

Fig. 6 A capillary blood vessel observed on the collagen fibers of the macula cribriformis. (B-stain) ×1000

〔F. Maki〕

Fig. 7 A nerve fiber running along a blood vessel of the diaphragmatic peritoneum (B-stain) ×400

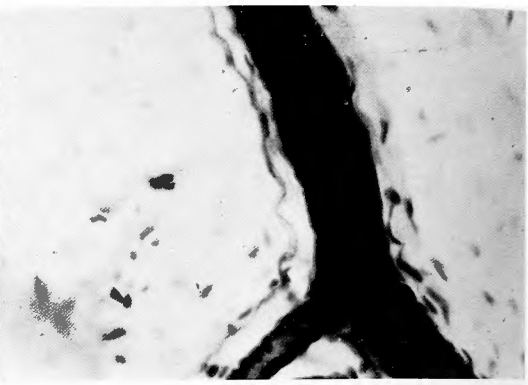

Fig. 8 A nerve fiber running along a blood vessel of the diaphragmatic peritoneum (B-stain) ×400

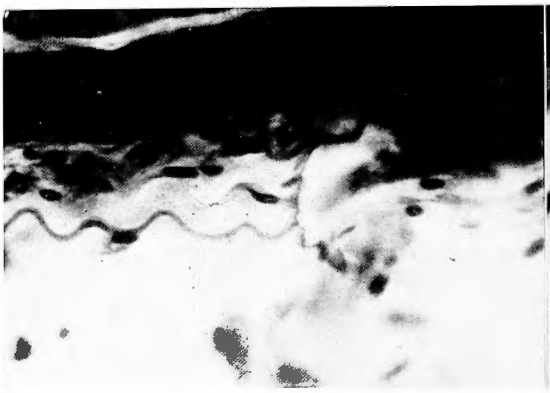

Fig. 9 Vegetative nerve elements forming so called nervous syncytium running along a blood vessel of the diaphragmatic peritoneum. (B-stain) ×400

Fig. 10 Enlargement of a part of Fig. 9. (B-stain) ×1000

Fig. 11 Enlargement of another part of Fig. 9, SCHWANN'S nuclei are scattered along its course. (B-stain) ×1000

Fig. 12 Nerve fibers running on the collagen fibers of the macula cribriformis separately from the blood vessels. (B-stain) ×400

〔F. MAKI〕

Fig. 13 The nervous syncytium distributed independently from that of blood vissels. Within the syncytium, SCHWANN's nucleus is shown. (B-stain) ×1000

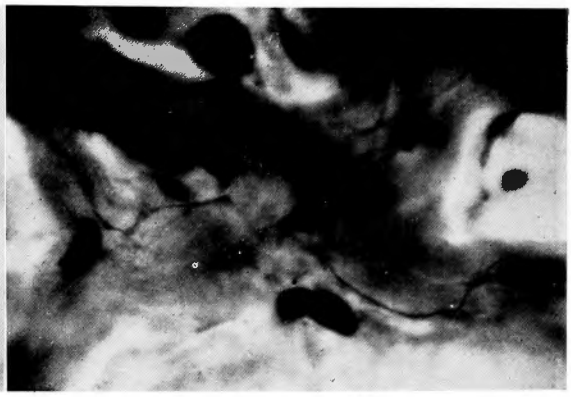

Fig. 14 Nerve fibers running on the collagen fibers of the macula cribriformis in the tendinous portion. Arborization to the terminal branches is shown. (B-stain) ×1000

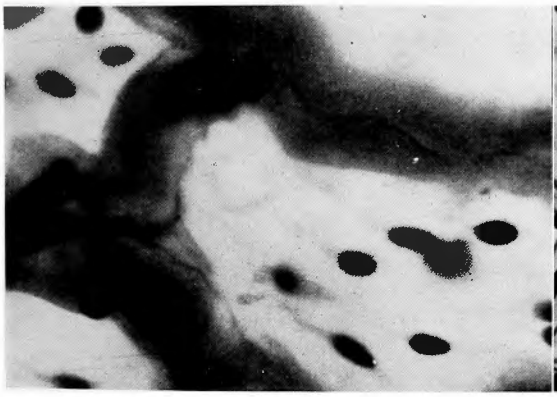

Fig. 15 Nerve fibers running on the collagen fibers of the macula cribriformis in the tendinous portion. (B-stain) ×1000

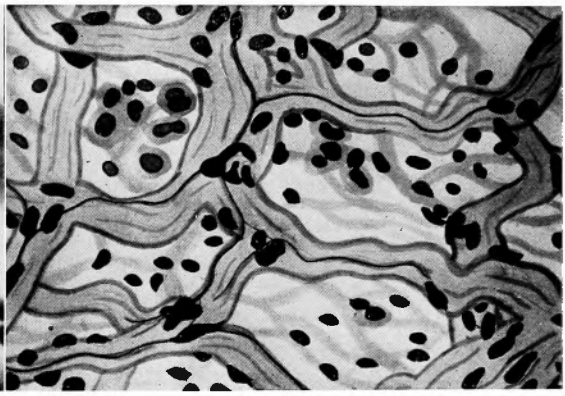

Fig. 16 A sketch showing the course nerve fibers running on the macula cribriformis in the tendinous portion.

Fig. 17 The nervous syncytium on the macula cribriformis in the border between the tendinous and the muscular portion. Within the syncytium, fine nerve fibers form the networks. (B-istain) ×1000

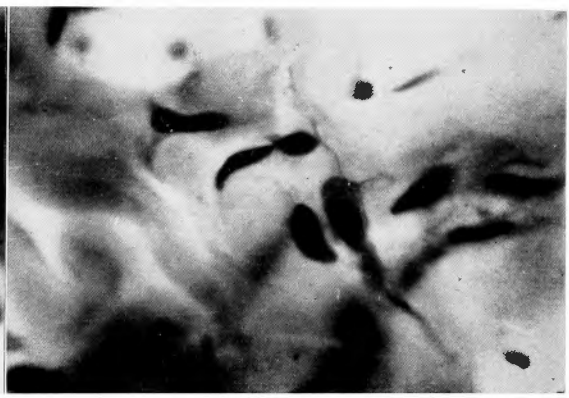

Fig. 18 A nerve fiber running together with fine terminal nervous network on the collagen fibers of the macula cribriformis. SCHWANN's nucleus lies in intimate relation with these nerve elements. (B-stain) ×1000 〔F. MAKI〕

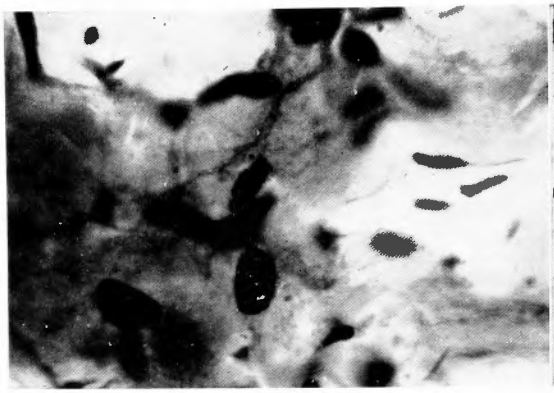

Fig. 19 Terminal structure of the nerve fibers on the collagen fibers of the macula cribriformis in the border between the tendinous and the muscular portion. Some end in free and some others form the fine networks. (B-stain) ×1000

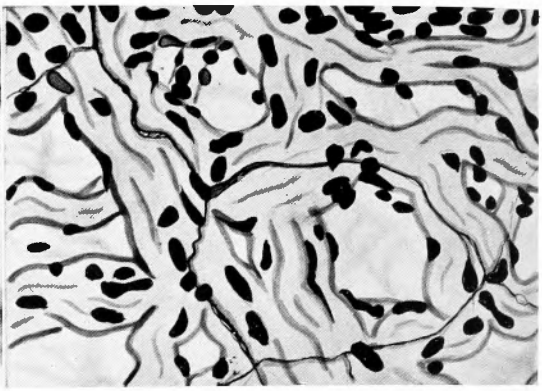

Fig. 20 A sketch showing the course of nerve fibers running on the collagen fibers of the macula cribriformis in the border between the tendinous and the muscular portion.

Fig. 21 A thick nerve fiber on the collagen fibers of the macula cribriformis in the muscular portion. (B-stain) ×1000

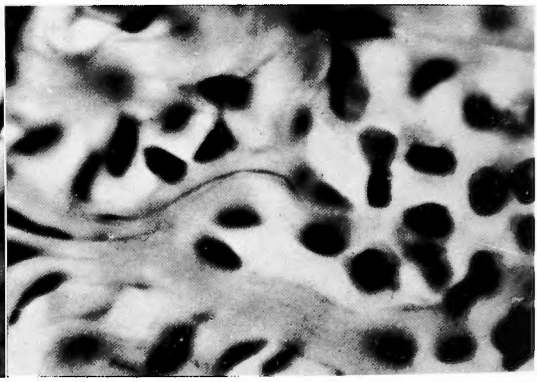

Fig. 22 Another nerve fiber in the same specimen. (B-stain) ×1000

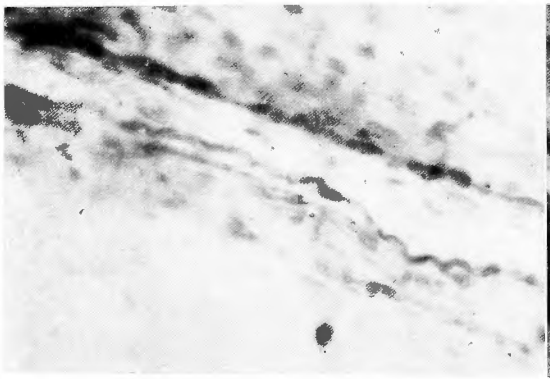

Fig. 23 A normal myelinated nerve fiber running along a blood vessel around the macula cribriformis. 7 days after section of the vagus nerve on one side. (E-stain) ×400

Fig. 24 A myelinated nerve fiber observed 6 days after section of the vagus nerve on one side. RANVIER-nodes are clearly shown. No sign of degeneration is shown (E-stain) ×400 〔F. MAKI〕

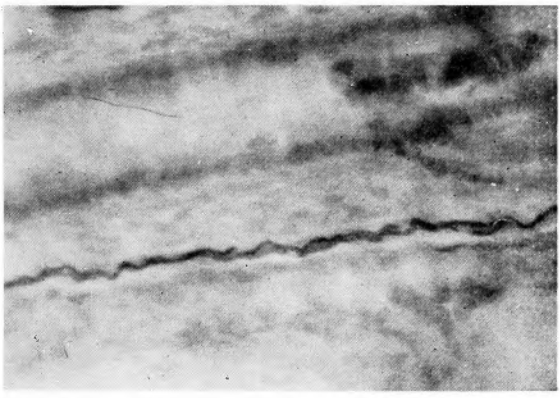

Fig. 25 A normal myelinated nerve fiber running along a blood vessel around the macula cribriformis. 6 days after section of the phrenic nerve on both sides. (E-stain) ×400

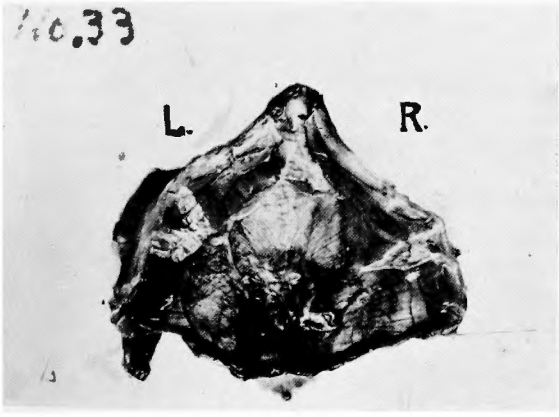

Fig. 26 The specimen was taken from the control group. India ink injected into the peritoneal cavity of the rabbit is filled somewhat more in the subpleural diaphragmatic lymphatics of the left side than the right.

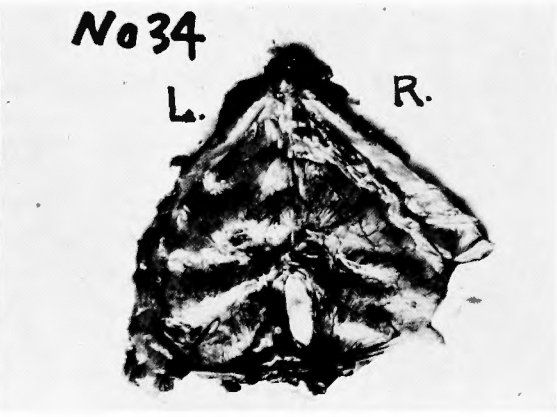

Fig. 27 The specimen was taken from the control group. India ink is filled somewhat more in the subpleural diaphragmatic lymphatics of the right side than the left.

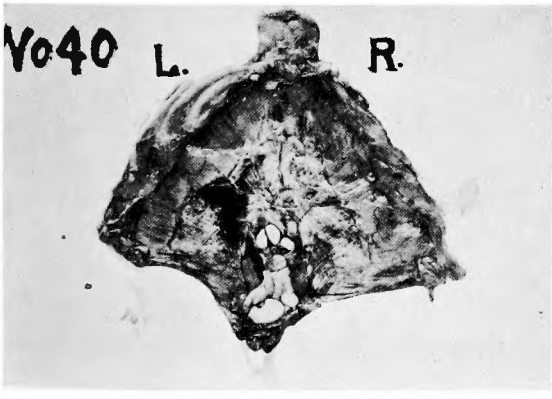

Fig. 28 The specimen was taken from the control group. India ink is filled to same degree in the subpleural diaphragmatic lymphatics of both sides.

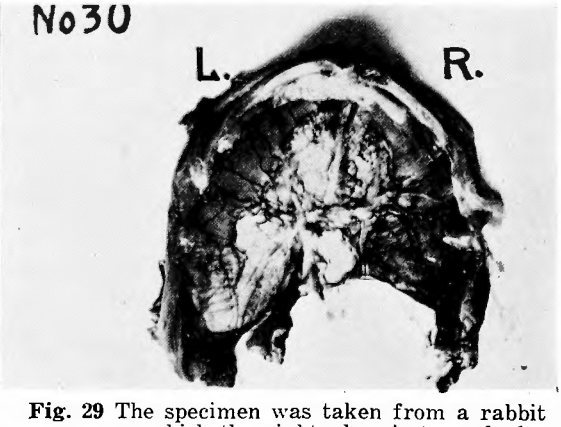

Fig. 29 The specimen was taken from a rabbit on which the right phrenicotomy had been performed 2 days before. India ink is filled to same degree in the subpleural diaphragmatic lymphatics of both sides. [F. MAKI]

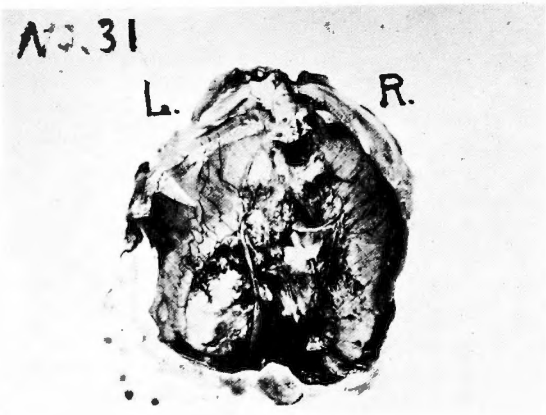

Fig. 30 The specimen was taken from a rabbit on which the right phrenicotomy had been performed 2 days before. India ink is filled somewhat more in the subpleural diaphragmatic lymphatics of the left side than the right.

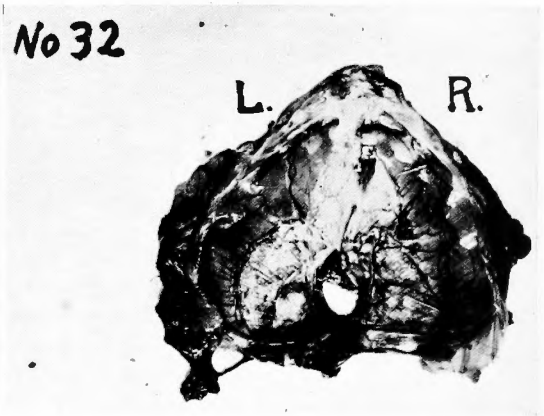

Fig. 31 The specimen was taken from a rabbit on which the right phrenicotomy had been performed 2 days before. India ink is filled somewhat more in the subpleural diaphragmatic lymphatics of the right side than the left.

EXPLANATION OF FIGURES

1) B-stain : BIELSCHOWSKY-SUZUKI's silver impregnation.
2) E-stain : EHRLICH's acid hematoxyline staining.

〔F. MAKI〕

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家兎横隔膜篩状斑の神経支配に関する研究

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1) Bielschowsky鈴木氏神経軸索染色法及び Ehrlich 氏神経髄鞘染色法を施した家兎横隔膜腹膜の伸展標本を検索して，前リンパ管通液路（木原教授）を構成する横隔膜篩状斑には，その膠原性線維に神経分布が存在することを認めた．更に，迷走神経及び横隔神経を切断した家兎について，篩状斑及びその附近の神経の2次変性を追求したが，明らかな神経の変性は観察されなかつた．

2) 偏側の迷走神経，横隔神経及び交感神経を切断した家兎の腹腔内に注入した墨汁が横隔膜の左右の篩状斑を経て吸収される度合を比較することによつて，篩状斑に分布する神経の機能を検討したが，此の実験においては，これらの神経切断が腹腔内異物吸収に及ぼす影響は観察されなかつた．